

The following listing of the claims will replace all prior versions and all prior listings of the claims in the present application

1. (currently amended) A method of diagnosing or prognosing a disease ~~in an individual~~ a test subject, comprising ~~the steps of~~:

for each gene of a collection of two or more genes:

a) ~~determining the level of expression of a gene in~~ detecting a presence, using an oligonucleotide of predetermined sequence, in RNA of a blood sample of an individual, and which has not been fractionated into cell types from said test subject, of RNA encoded by said gene, wherein said gene is expressed in blood and in a non-blood tissue of a subject who is not said test subject, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene in said sample;

(b) quantifying a level of said RNA encoded by said gene; and

~~b) c) determining~~ detecting a difference between ~~of said quantified level of expression of said gene in said blood sample according to step a) relative to the~~ and a quantified level of expression of the same gene of a control RNA encoded by said gene in RNA of blood samples which have not been fractionated into cell types, from control subjects not having said disease, said control RNA having been detected in said samples from said control subjects, wherein a difference in expression levels is indicative or predictive of

thereby diagnosing or prognosing said disease in said test subject.

2. (original) A method of diagnosing or prognosing a disease in a test subject, said method ~~an individual~~, comprising ~~the steps of~~:

for each gene of a collection of two or more genes:

a) producing an amplification product from RNA of ~~determining the level of expression of a gene in a blood sample of an individual~~ which has not been fractionated into cell types from said test subject, using primers specific only for RNA and/or cDNA complementary to said

RNA, encoded by said gene in said sample, wherein said gene is expressed in blood and in a non-blood tissue of a subject who is not a test subject; and

b) quantifying a level of said amplification product; and

c) b) determining a difference between said quantified level of said amplification product and a quantified level of amplification products produced using said primers from control RNA of blood samples which have not been fractionated into cell types, from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby diagnosing or prognosing said disease in said test subject

~~detecting the same level of expression of said gene in said blood sample according to step a) relative to the level of expression of the same gene of a control, wherein the same level of expression is indicative or predictive of said disease.~~

3. (Canceled)

4. (Canceled)

5. (Canceled)

6. (Canceled)

7. (Currently amended) A method of identifying a marker useful for diagnosing a disease, said method comprising the steps of:

a) using an oligonucleotide of predetermined sequence, detecting a the presence in RNA of blood samples which have not been fractionated into cell types from subjects having said disease, of RNA encoded by a gene, said gene expressed in blood and in a non-blood tissue of a subject not having said disease, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene in said samples in an unfractionated sample of whole blood from each of one or more subjects having said disease, and

b) quantifying a level of said RNA encoded by said gene in said sample; and

b) c) determining a difference between said quantified level and a quantified level of control RNA encoded by said gene in RNA of from an unfractionated sample of whole blood samples which have not been fractionated into cell types, from each of one or more first control subjects, said control RNA having been detected being encoded by said gene and being detectable in said samples from said control subjects,

thereby said difference identifying said gene as being a marker useful for diagnosing of said disease.

8. (Currently amended) A method of identifying a marker useful for diagnosing a disease, said method comprising the steps of:

a) producing an amplification products from RNA of blood samples which have not been fractionated into cell types, extracted from an unfractionated sample of whole blood from each of one or more subjects having said disease, said using primers specific only for RNA, and/or cDNA complementary to said RNA, amplification product being encoded by a gene in said samples, said gene being expressed in blood and in a non-blood tissue of a subject not having said disease, and quantifying said amplification product; and

b) quantifying a level of said amplification products; and

c) determining a difference between said quantified level quantity of said amplification products and a quantified level quantity of an amplification products produced using said primers from control RNA, in RNA of blood samples which have not been fractionated into cell types said control RNA being extracted from an unfractionated sample of whole blood from each of one or more control subjects, said amplification product from said control RNA being encoded by said gene, and expression of said gene being detectable having been detected in said samples from said each of one or more control subjects, thereby identifying said gene as being a marker useful for diagnosing wherein a said difference identifies said gene as a marker of said disease.

9. (canceled)

10. (canceled)

11. (canceled)

12. (canceled)

13. (canceled)

14. (canceled)

15. (canceled)

16. (canceled)

17. (Currently amended) A The method of identifying two or more markers useful for diagnosing a disease, said method ~~claim 7, further~~ comprising:

for each of a collection of two or more genes:

a) using an oligonucleotide of predetermined sequence (e) in said sample of step (a):
detecting a presence, in RNA of blood samples which have no been fractionated into cell types
from subjects having said disease of an RNA encoded by a second said gene, said
oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by
said gene in said samples, said second gene being distinct from said gene in step (a) and being
expressed in blood and in a non-blood tissue of said a subject not having said disease;

b) ~~and~~ quantifying a level of said RNA encoded by said ~~second~~ gene; and

~~(d)c)~~ determining a difference between said quantified level and a quantified level of
control RNA encoded by said gene in RNA of blood samples which have not been fractionated
into cell types from control subjects, said control RNA having been detected, in an
unfractionated sample of whole blood from each of one or more second control subjects, a
difference between: said level of said RNA encoded by said second gene, and a quantified level
of a control RNA being encoded by said second gene, and being detectable in said samples from

said second control subjects, and wherein said difference identifying between: said level of said RNA encoded by said second gene; and said quantified level of said control RNA being encoded by said second gene identifies said second gene as a further marker of said disease,

thereby identifying said two or more markers as useful for diagnosing said disease.

18. (canceled)

19. (currently amended) A The method of identifying two or more markers useful for diagnosing a disease, said method claim 8, further comprising:

for each of a collection of two or more genes:

(e)a) producing amplification products from said RNA of blood samples which have not been fractionated into cell types from subjects having said disease, using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by said gene of said subjects, wherein said gene step (a) an amplification product encoded by a second gene, said second gene being distinct from said gene of step (a) and being is expressed in blood and in a non-blood tissue of said a subject not having said disease; and

b) quantifying a level of said amplification products; and encoded by said second gene;
and

(d)c) determining a difference between said quantified level a quantity of said amplification products of step (e) and a quantified level quantity of an amplification products produced using said primers, from control from reference RNA, in RNA of blood samples which have not been fractionated into cell types, from control subjects, said reference RNA being extracted from an unfractionated sample of whole blood from each of one or more reference subjects, said amplification product from reference RNA being encoded by said second gene, and expression of said control RNA having been detected second gene being detectable in said samples from said each of one or more reference control subjects, wherein said difference of step (d) identifies said second gene as a further marker of said disease,

Thereby identifying said two or more markers as useful for diagnosing the disease.

20. (currently amended) The method of any one of claims 1, 7, and 17, wherein said detecting of said RNA encoded by each said gene of step (a) is effected by detecting cDNA and/or EST derived from said RNA of step (a).

21. (currently amended) The method of any one of claims 2, 8, and 19, wherein said producing of said amplification products ~~of said RNA~~ of step (a) is effected by producing an amplification products from ~~of~~ cDNA and/or EST derived from said RNA encoded by each said gene of step (a).

22. (canceled)

23. (Currently amended) The method of any one of claims 1, 7, and 17, further comprising:
(e) ~~—quantifying a level of said control RNA in said sample of step (b) to thereby~~
determine said quantified level of said control RNA.

24. (Currently amended) The method of any one of claims 2, 8 and 19, further comprising:
~~(e) —quantifying a level of said amplification products from said control RNA in said~~
~~sample of step (b) to thereby determine said quantity~~ quantified level of said
amplification products produced from said control RNA.

25. (canceled)

26. (canceled)

27. (canceled)

28. (Currently amended) The method of any one of claims 1, 2, ~~claim 7~~, 8, 17 and 19, wherein said quantifying of said level of said RNA in step (b) ~~of step (a)~~ is effected by determining a quantity of said RNA ~~of step (a)~~ relative to a ~~quantity~~ of a housekeeping gene.

29. (Currently amended) The method of any one of claims ~~claim~~ 1, 7 and 17, wherein said quantified ~~quantifying of said~~ level of said control RNA ~~is effected by determining a quantity of~~ has been determined relative to ~~a quantity of~~ a housekeeping gene.

30. (canceled)

31. (currently amended) The method of any one of claims 2, 8 and 19, wherein said quantified level ~~quantifying of said~~ amplification products produced from said control RNA has been determined ~~is effected by determining a quantity of said amplification product from said control~~ RNA relative to ~~a quantity of~~ a housekeeping gene.

32. (previously presented) The method of claim ~~[[7 or 8]]~~ 1 or 2, wherein said test subject ~~each of said one or more subjects having said disease, said subject not having said disease and/or each of said one or more control subjects~~ is a human.

33. (currently amended) The method of any one of claims ~~claim~~ 1, 2, 7, 8, 17 and 19, wherein said ~~each of said one or more subjects having said disease, said subject not having said disease, said each of said one or more first control subjects and/or said each of said one or more second control subjects~~ is a ~~are~~ human.

34. (currently amended) The method of any one of claims ~~claim~~ 1, 2, 7 or 8, 17 and 19, wherein none of said ~~one or more~~ control subjects have said disease.

35. (canceled)

36. (canceled)

37. (canceled)

38. (New) The method of claim 2, 8 or 19, wherein said producing of said amplification products of step (a) is effected by producing amplification products from cDNA and/or EST derived from said RNA of step (a).

39. (New) The method of claims 7, 8, 17 or 19, wherein said subjects having said disease have no overt symptoms with respect to said disease.

40. (New) The method of claim 7, 8, 17 or 19, wherein said subjects having said disease are human.

41. (New) The method of claim 1, or 17, wherein said quantifying of said level of said RNA encoded by each said gene of step (a) is effected by quantifying a level of cDNA and/or EST derived from said RNA encoded by each said gene of step (a).

42. (New) The method of claim 7, wherein said quantified level of said RNA encoded by said gene of step (a) is effected by quantifying a level of cDNA and/or EST derived from said RNA encoded by said gene of step (a).

43. (New) The method of claim 1, 2, 7, 8, 17 or 19 wherein said disease is selected from the group consisting of colorectal cancer, diabetes, and heart failure.

44. (New) The method of claim 1 or 2, wherein said test subject has not previously been diagnosed with said disease.

45. (New) The method of claim 1 or 2, wherein said test subject is asymptomatic with respect to said disease.

46. (New) The method of claim 7, 8, 17 or 19, wherein said subjects having said disease are asymptomatic with respect to said disease.

47. (New) The method of claim 1 or 2, wherein said gene is a marker of disease progression.

48. (New) The method of claim 1 or 2, wherein said test subject has no overt symptoms with respect to said disease.

49. (New) The method of claim 7, 8, 17 or 19, wherein said control subjects have said disease at a different stage than said subjects having said disease.

50. (New) A method of diagnosing or prognosing a disease in a test subject, comprising:
for each gene of a collection of two or more genes:

(a) detecting a presence, using an oligonucleotide of predetermined sequence, in RNA of unfractionated cells of a lysed blood sample, from said test subject, of RNA encoded by said gene, wherein said gene is expressed in blood and in a non-blood tissue of a subject who is not said test subject, and said oligonucleotide is specific only for RNA, or cDNA complementary to said RNA, encoded by said gene;

(b) quantifying a level of said RNA encoded by said gene in said sample; and

(c) determining a difference between said quantified level and a quantified level of control RNA encoded by said gene in RNA of unfractionated cells of lysed blood samples, from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby diagnosing or prognosing said disease in said test subject.

51. (New) A method of diagnosing or prognosing a disease in a test subject, said method comprising:

for each gene of a collection of two or more genes:

(a) producing an amplification product from RNA of unfractionated cells of a lysed blood sample from said test subject, using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by a gene in said sample, wherein said gene is expressed in blood and in a non-blood tissue of a subject who is not said test subject;

(b) quantifying a level of said amplification product; and

(c) determining a difference between said quantified level of said amplification product and a quantified level of amplification products produced using said primers from control RNA of unfractionated cells of lysed blood samples, from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby diagnosing or prognosing said disease in said test subject.

52. (New) A method of identifying a marker useful for diagnosing a disease, said method comprising:

(a) using an oligonucleotide of predetermined sequence, detecting a presence in RNA of unfractionated cells of lysed blood samples from subjects having said disease, of RNA encoded by a gene expressed in blood and in a non-blood tissue of a subject not having said disease, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by a gene in said samples;

(b) quantifying a level of said RNA encoded by said gene; and

(c) determining a difference between said quantified level and a quantified level of control RNA encoded by said gene in RNA of unfractionated cells of lysed blood samples, from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby identifying said gene as being a marker useful for diagnosing said disease.

53. (New) A method of identifying a marker useful for diagnosing a disease, said method comprising:

(a) producing amplification products from RNA of unfractionated cells of lysed blood samples from subjects having said disease, using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by a gene in said samples, said gene being expressed in blood and in a non-blood tissue of a subject not having said disease;

(b) quantifying a level of said amplification products; and

(c) determining a difference between said quantified level of said amplification products and a quantified level of amplification products produced using said primers from control RNA in RNA of unfractionated cells of lysed blood samples, from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby identifying said gene as being a marker useful for diagnosing said disease.

54. (New) A method of identifying two or more markers useful for diagnosing a disease, said method comprising:

for each gene of a collection of two or more genes:

(a) using an oligonucleotide of predetermined sequence, detecting a presence, in RNA of unfractionated cells of lysed blood samples, from subjects having said disease, of RNA encoded by said gene, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene in said samples, and said gene being expressed in blood and in a non-blood tissue of a subject not having said disease;

(b) quantifying a level of said RNA encoded by said gene in said samples; and

(c) determining a difference between said quantified level and a quantified level of control RNA encoded by said gene in RNA of unfractionated cells of lysed blood samples, from control subjects, said control RNA having been detected in said samples from said control subjects, and said difference identifying said gene as a marker of said disease,

thereby identifying said two or more markers useful for diagnosing said disease.

55. (New) A method of identifying two or more markers useful for diagnosing a disease, said method comprising:

for each gene of a collection of two or more genes:

(a) producing amplification products from RNA of unfractionated cells of lysed blood samples from subjects having said disease, using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene of said subjects, wherein said gene is

expressed in blood and in a non-blood tissue of a subject not having said disease;

(b) quantifying a level of said amplification products; and

(d) determining a difference between said quantified level of said amplification products and a quantified level of amplification products produced using said primers from control RNA in RNA of blood samples which have not been fractionated into cell types, from control subjects, said control RNA having been detected in said samples from said control subjects, wherein said difference identifies said gene as a marker of said disease.

thereby identifying two or more markers useful for diagnosing the disease.